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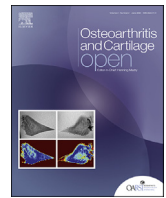
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Evaluation of serum ARGS neopeptide as an osteoarthritis biomarker using a standardized model for exercise-induced cartilage extra cellular matrix turnover

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SUMMARY

Objective: To propose a standardized model for exercise-induced cartilage turnover and investigate residual levels and dynamics of biomarker serum ARGS (sARGS) in primary osteoarthritis (OA) patients and a supportive group of young healthy subjects.

Method: The trial is a randomized, cross-over, exploratory study with interventions of exercise and inactivity. 20 subjects with knee OA, as well as 20 young healthy subjects (mean age 25.7 years (range; 19–30), 50% male), underwent cycling, running and resting interventions on separate days one week apart. Blood samples were taken at baseline, immediately, 1, 2, 3 and 24 h after activity start. sARGS was measured by sandwich ELISA.

Results: Intraclass correlation between visits were 0.97 and 0.77 for the OA and healthy group, respectively. An acute drop in sARGS in response to high-intensity exercise was observed in both groups. Minute acute sARGS increase was observed in OA subjects in response to moderate intensity running and cycling, which normalized within 24 h. In healthy subjects an acute drop in sARGS was seen immediately after running, but not cycling, and no other changes were observed. A negative correlation between baseline Kellgren-Lawrence (KL) grade and baseline sARGS ($r = -0.69$, $p = 0.002$) in OA was found. A negative correlation between age and sARGS was found in healthy subjects ($r = -0.67$, $p = <0.002$).

Conclusion: sARGS sensitivity to physical activity is considered low and sARGS is a reproducible and stable marker. Minute acute increases in sARGS were observed in the hours following moderate intensity exercise.

1. Introduction

Change of life-style is known to be beneficial [1] and in general physical activity is recommended to maintain mobility in Osteoarthritis (OA) [2]. Optimal loading of cartilage may promote anti-catabolic and anabolic effects in cartilage [3,4]. Yet, the acute impact of different exercise programs remains to be evaluated, although the joint load, and thus the effect, of running vs. cycling is considered different, and may

present different clinical utility.

Joint specific biochemical markers have become important tools in OA research and development, where they are usually applied in the investigation of disease modifying OA drug (DMOAD) candidates. Here they may be used to optimize study populations [5], evaluate progression [6] as well as treatment response [7]. Potential use also includes early detection of OA [8], assessment of cartilage receptiveness to load [9], determination of safe forms of exercise [10] and ability to adapt [11]. It

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is, however, crucial for the applicability of biochemical markers to establish their significance. Thus, biochemical markers must be characterized and validated [12].

DMOAD trials are costly and time-consuming large studies [13]. A low-cost model for rapid assessment would therefore be advantageous. Such a model could evaluate potential chondroprotective properties of DMOAD, based on changes in evoked cartilage turnover after joint-load. To our knowledge, no standardized human model for assessment of evoked cartilage turnover has previously been described.

Biochemical markers of cartilage turnover, such as cartilage oligomeric matrix protein (COMP), have been widely studied and are known to increase following weight-bearing exercise in healthy individuals [10]. A few studies, heterogeneous in design, investigated the biochemical marker's response in OA subjects following exercise [14–17], all of which acutely increased serum COMP. Furthermore, studies found mechanically induced changes in biomarkers to be associated with cartilage thinning after 5 years [14,18].

Aggrecan is a major component of articular cartilage extra cellular matrix (ECM) [19], but may to a lesser extent be present in ECM of other organs [20], such as veins [21]. Increased turnover is expected in response to load stress, and subsequent changes in serum concentrations of aggrecan fragments may therefore reflect the degree of induced cartilage turnover. A fragment of aggrecan, the ARGS neopeptide, has previously been proposed as a promising prognostic and pharmacodynamic marker in OA drug development [22].

The ARGS neopeptide is generated enzymatically by A disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) [23] and is thought to reflect cartilage turnover [24]. ARGS has primarily been investigated in synovial fluid (SF) of knee trauma subjects, where it has been observed to increase after injury [25–27]. Levels of ARGS in SF are also increased in knees affected by OA [26] as compared to healthy individuals. However, concentrations in SF can [25], but may not always reflected in serum [22,28]. Serum ARGS (sARGS) has been investigated in a single cross-sectional study including OA subjects [22] and was found to be associated with OA disease severity and age, while the dynamics of sARGS in OA still represents a gap in the literature.

The objectives of this exploratory pilot study were to propose a standardized model for exercise-evoked cartilage turnover and investigate sARGS dynamics, residual levels and changes in response to exercise of various mechanical loading in human knee OA subjects and a supportive group of young healthy individuals. We aimed to evaluate the feasibility of the model and, further characterize sARGS as a biomarker of cartilage turnover in OA.

2. Methods

2.1. Study design

This pilot study was an exploratory, randomized, cross-over trial with exercise intervention of OA subjects and a supportive group of young healthy individuals. Participants from both groups underwent two active interventions in a randomized order followed by a third “resting” intervention. Study visits were scheduled with a one week interval to facilitate “wash-out” of circulating biomarkers and participants were instructed be

minimally active (e.g. no biking to the site) and to fast 6 h prior to visits. For the purpose of conducting exercise-interventions at a comparable intensity for all subjects, the maximal heart rate (HR_{max}) was determined by HR_{max} -test performed prior to the first intervention. The outline of study interventions is shown in Fig. 1.

2.2. Subjects

Twenty OA subjects and twenty healthy subjects were planned to be enrolled. Subjects were recruited through advertisements on social media and in the local paper. Inclusion criteria for the OA group were x-ray verified Kellgren-Lawrence grade 1–3 in a least one tibio-femoral joint, age 35–75 years and body mass index (BMI) between 18.5 and 35 kg/m². Exclusion criteria were previous knee or hip arthroplasty, arthroscopy or intra-articular injections of therapeutic agents within the last 6 months, presence of autoimmune disease or secondary OA, treatment with beta-blockers, corticosteroids or anticoagulants, active systemic disease and history of cardiovascular disease, alcoholism/drug abuse.

Inclusion criteria for healthy subjects were age 18–75 years, KL 0 and BMI between 18.5 and 35 kg/m². Exclusion criteria were osteoarthritis, suspicion of osteoarthritis, previous knee or hip arthroplasty, arthroscopy or intra-articular injections within 6 months, treatment with beta-blockers, corticosteroids or anticoagulants, active systemic disease and history of cardiovascular disease, alcoholism/drug abuse.

2.3. Procedures

At the screening visit demographics data, standard medical history and physical activity history was obtained. Physical exam including vital signs, ECG and bilateral knee x-ray was performed and subjects filled in the KOOS Pain subscale (P1–P9).

A supervised HR_{max} -test was carried out on a spinning bike at visit two during the screening period to experimentally establish the accurate HR_{max} . The heart rate (HR) was monitored using a pulse belt and during the entire test the investigator encouraged and motivated the subject in order to reach the highest possible HR. Upon establishment of HR_{max} , meeting all inclusion criteria and no exclusion criteria, the subjects were randomized to a sequence of interventions as illustrated in Fig. 1. We aimed to assess the difference between the experimental and calculated (theoretical) HR_{max} for future reference.

Instructions were given to be minimally physically active on the day before and the day of intervention, both active and resting. On days of active intervention, subjects were instructed to meet after fasting for 6 h and maintain fasting until all samples were taken. Only intake of water was allowed during fasting. Subjects were equipped with a heart rate monitor and a blood sample was taken a baseline (approximately 5 min before initiation of activity). Running was carried out on a treadmill and cycling was carried out on a spinning bike. Activity was initiated with a 10 min warm-up walk at a HR below 75% of HR_{max} before running. Cycling was initiated with a 10 min cycling warm-up at a HR below 75% of HR_{max} . After the warm-up the intensity running speed and tread load, respectively, was regulated in order to quickly reach and maintain a HR of 75% of HR_{max} for 15 min. A cool-down of 5 min of walking and

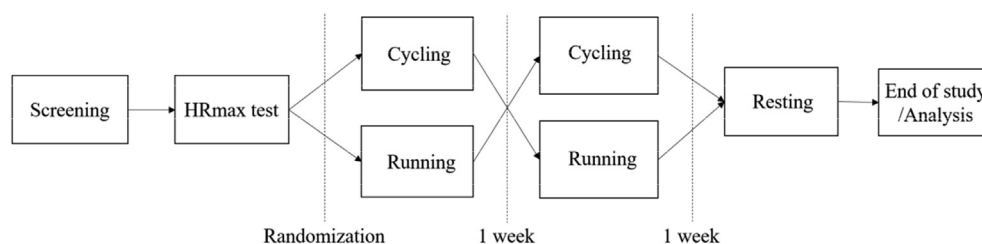


Fig. 1. Overview of the main study procedures.

cycling, respectively, aiming to lower the HR ended the bout. A blood sample was taken immediately after activity and 1, 2, 3 and 24 h after intervention start. If participants were not able to complete the running intervention, a rescue intervention of up-tempo walking with a similar protocol was applied. The subjects were seated in the clinic lounge area during the entire resting intervention and during the 2.5 h sampling period after exercise. Blood samples were taken 5 times with the same interval as during active intervention.

2.4. Recent physical activity history

Recent physical activity data was collected using a custom made semi-qualitative instrument. Significant cardio work-out was defined as an average of more than two weekly hours of self-reported significant cardio work-out during the previous two months from screening. Self-reported weekly hours and the type of significant work-out was recorded.

2.5. X-ray

X-ray images of both knees including femoral and tibial metaphyses and proximal fibula were obtained to assess the KL grading and to rule out significant knee pathology. A Syna-Flex™ device was used for consistent positioning. Subjects were positioned with even weight-distribution on both legs, flexing the knees until having the thighs in touch with Syna-Flex™.

Images were analyzed by an experienced radiologist.

2.6. KOOS pain

KOOS is a self-explanatory questionnaire for assessment of the patient's opinion about their knee problems considering the previous week as the time period [29]. KOOS is also commonly used to assess groups in knee OA research [29]. For each question, the subject responds on a 5-point likert scale and assigned a score from 0 to 4. For data analysis, the score is normalized using the formula: KOOS Pain = 100 - (Mean score x 100/4). Thus, a score of 100 indicates no symptoms and 0 indicates extreme symptoms.

2.7. ARGs neoepitope biomarker assay

The ARGs marker was measured using a chemiluminescence sandwich assay (huARGs), applying the monoclonal antibodies F78 against the G2 domain and OA-1 targeting ARGs neoepitope was used as a detection antibody [22]. In brief, a 96-well streptavidin pre-coated microplate was coated with biotinylated F78 for 30 min at 20 °C on a shaker. Following five washes with wash buffer (20 mM Tris, 50 mM NaCl, pH 7.2), 20 µL standards, controls or samples were added to appropriate wells, followed by 100 µL of peroxidase labelled-OA-1 and the plate was incubated overnight (20 ± 1 h) at 4 °C. After washing, 100 µL of chemiluminescence substrate was added and the plate was read at a standard chemiluminescence reader. The detection range of the assay was 41–2523 pmol/mL. Two kit controls and one in-house controls were included where inter-assay CVs were <12%. Samples were run in duplicates and the median (range) intra-assay CV was 2.3 (0.1–17.6). Samples with duplicate CVs above 15% were rerun.

2.8. Statistical analysis

No formal power calculations were performed to assess the sample size in this exploratory study. The proposed sample size of 20 subjects in each group was deemed sufficient for exploratory analysis of biomarker changes. The two groups were investigated using the same method, but not intended for comparison, and thus analyzed separately.

Missing values (i. e. due to lost samples) were registered and reported, but were left out of analyses.

Baseline ARGs from samples, defined as the first set of samples taken,

from each intervention visit (running, cycling and resting) were used to assess the inter-day variability of OA subjects and healthy volunteers under standardized conditions (minimal physical activity and fasting). Interday variability was calculated and assessed by repeated single measures intra-class correlation coefficient (ICC). For correlation analyses “baseline ARGs” was defined as the sARGs concentration from the first sample taken during rest and fasting.

Statistical significance of sARGs changes following HR_{max}-test was assessed by paired T-test.

Mean proportional changes in sARGs from baseline and standardized to resting values, respectively, were calculated and plotted with 95% confidence intervals (CI).

The area under the curve (AUC) for time interval 0–3 h for each intervention was calculated and evaluated by their 95% CI.

Multiple regression was used to examine factors influencing sARGs. We adjusted for age, sex, BMI, KL grade (both highest and cumulated) where applicable.

Statistical analyses were performed using MedCalc version 18.11.6. GraphPad Prism 8 was used for graphs and associated calculations, such as AUC and Spearman correlation.

2.9. Permissions

Informed consent was obtained from all participants prior to enrollment and data collection.

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. The study was approved by National Ethical Committee (approval number: H-18038807). The study was funded by Nordic Bioscience and the Danish Research Foundation.

3. Results

3.1. Study overview

48 subjects were screened and 40 were randomized, resulting in 20 subjects in each group as planned. All enrolled subjects completed the study. One subject was excluded from analysis as sARGs in this subject was below the lower limit of the quantifiable range in all samples. A total of four single samples were lost, one in the OA group and three in the healthy group. The one lost sample from the OA group was a 3 h sample after cycling. The three lost samples in the healthy group were all 24 h follow-up samples – one after cycling and two after running. The overview of study populations is displayed in Fig. 2 and baseline characteristics are displayed in Table 1.

The experimentally measured HR_{max} and the theoretical HR_{max} differed 0.2% (SD: 5.9) in average, with the theoretical HR_{max} calculated by the formula: HR_{max} = 206.3*(0.711*age) [30]. As shown in Fig. 3, a statistically significant decrease in the level of sARGs was found immediately after the HR_{max}-test in both the OA (p < 0.05) and the healthy group (p < 0.001).

The mean sARGs changes from baseline were calculated for each intervention in both groups and listed in Table 2.

3.2. Technical performance of the ARGs assay

Technical performance of ARGs assay: Intra- and inter-assay variations were 1.7–2.1% and 4.8–9.6%, respectively. The lower limit of detection was 25.7 pM and the quantifiable range was between 83.1 and 1329 pM.

The interday-variability was relatively low, particularly in the OA group, as displayed in Fig. 4a–b, and the ICC, which is ideally 1.0, was calculated as 0.97 (95% CI: 0.94–0.99) for OA subjects and 0.77 (95% CI: 0.57–0.89) for healthy subjects.

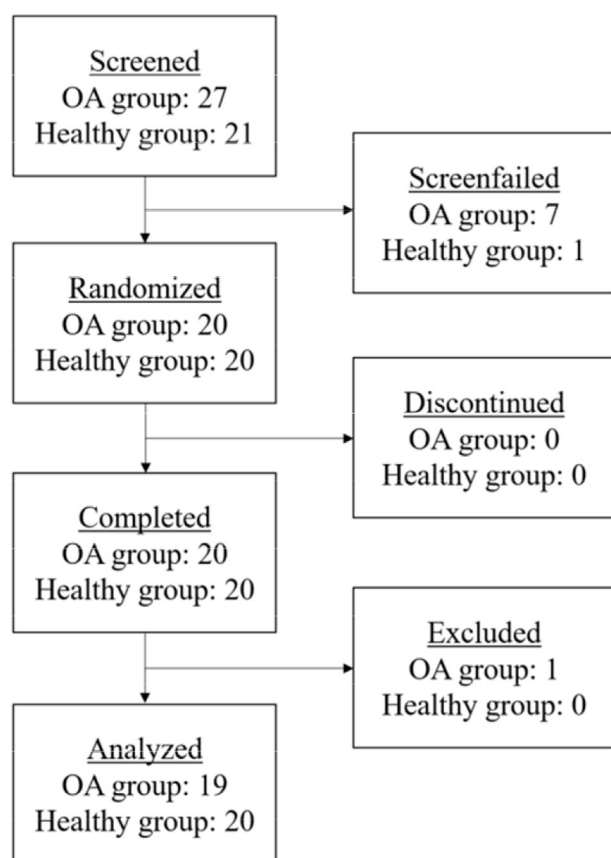


Fig. 2. CONSORT chart of the study populations and enrollment. Two OA subjects were screen-failed due to post-traumatic OA and five were screen-failed due to x-ray verified KL grade 4. One healthy subject was screen-failed due to x-ray verified OA. One OA subject was excluded from analysis, as sARGS was below the lower limit of the quantifiable range in all samples.

Table 1
Baseline characteristics for study subjects included for analysis.

	OA subjects (N = 19)	Healthy subjects (N = 20)
Mean age, years (R)	61.9 (9.1)	25.7 (3.82)
Male sex, n (%)	5 (26.3)	10 (50)
Mean BMI (kg/m ²) (SD)	26.3 (3.4)	22.5 (1.6)
Highest KL-grade at baseline, n (%)	1: 3 (15.8) 2: 7 (36.8) 3: 9 (47.4)	0
Mean ARGs (pmol/L) (SD)	348 (209)	428 (93.6)
Mean KOOS Pain (SD)	74.4 (11.4)	95.1 (8.1)

3.3. Subjects with osteoarthritis

Twenty subjects completed the cycling and resting interventions and provided measurable levels of sARGS, 15 subjects completed running, while 5 subjects completed the rescue intervention instead of the running intervention.

As shown in Fig. 5a+b, mean sARGS trended to decrease from baseline shortly after exercise and subsequently increased one, two and 3 h after initiation of exercise. sARGS levels returned completely to baseline levels the day after cycling, but appeared slightly elevated the day after running. sARGS initially decreased and returned to baseline levels during 3 h of rest. The magnitude of the observed changes was generally small, and did not exceed 6.7% at any time point. Standardizing sARGS to resting levels appeared to accentuate the trend of increase, as seen on Fig. 5b. The area under the curve for the time interval 0–3 h was calculated for each graph in Fig. 5a + b, but none of the AUCs were statistically

significant from baseline or from the other interventions.

A negative correlation between cumulated KL grade and baseline sARGS ($r_{\text{partial}} = -0.69$, $p = 0.002$) (without the single outlier, $r_{\text{partial}} = -0.62$, $p = 0.01$), and a positive correlation with age and baseline sARGS ($r_{\text{partial}} = 0.67$, $p = 0.003$) (without the single outlier, $r_{\text{partial}} = 0.27$, $p = 0.33$) was found.

No statistically significant correlations were found between sex, physical activity history or pain and sARGS.

3.4. Healthy subjects

20 healthy participants completed the interventions.

As shown in Fig. 5c and d, sARGS decrease significantly shortly after running, but not after cycling. Subsequently sARGS trended to rise above baseline levels after two and 3 h, respectively. sARGS returned to baseline levels the day after running, but was still elevated the day after cycling. sARGS trended to increase during 3 h of rest.

AUCs for Fig. 5c and d were calculated and compared by their 95% CIs. None of the AUCs were significantly different from 0 or from the other AUC of the other interventions.

A negative correlation was found between age and baseline ARGs, adjusted for covariates ($r_{\text{partial}} = -0.67$, $p = 0.002$). The univariate correlation is plotted in Fig. 6.

No statistically significant correlations were found between sex, physical activity history or pain and sARGS.

4. Discussion

The existing literature on the impact of exercise on OA biomarkers is sparse, while healthy subjects have been widely studied [10]. This study applied a proposed model for evaluation of acute cartilage turnover after exercise and we report novel data on sARGS acute dynamics in human subjects.

4.1. Osteoarthritis

The overall results of this pilot study indicate that 1) the model is feasible for knee OA subjects, 2) sARGS sensitivity to physical activity is low, 3) sARGS is a valid reproducible marker as judged by the ICC of 0.97 reflecting minimal interday variability comparable to previous research on like biomarkers [31]. 4) A relatively large difference in the variation of baseline aggrecan turnover between subjects was observed, and the turnover rate remained stable for each subject throughout the study 5) a transient drop in sARGS is seen immediately after exercise which may be inversely proportional to exercise intensity 6) the results suggest a correlation between mechanical load and sARGS elevation, although the proportions of increase was low considering the load intensity. sARGS may continue to rise after the last sampling point 3 h after initiation of the exercise intervention, but was not sampled in the period between 3 h and approximately 24 h after initiation of exercise.

A higher cumulated KL grade correlated with a lower sARGS level. A possible explanation may be that a lower volume of cartilage results in lower ARGs release. Previously, sARGS has been observed to positively correlate with KL grade, which is contradictory [22]. However, the methodological differences, including structural severity, assessment of function and pain and OA subtype (only primary OA in this study) of the present and referred study limits the possibilities for direct comparison, and the reason for this discrepancy is unknown. The positive correlation between sARGS and age, and the absence of gender-based differences observed are in line with results from the other existing study on sARGS in human OA [22].

ADAMTS-5 is increased in human OA cartilage [32] and fragments of aggrecan including ARGs have previously been proposed as an early marker of OA progression [22,33]. Our results support that sARGS reflect aggrecan-, and thus presumably, cartilage turnover [33], although the potential contribution from cardio-vascular system is unknown. In

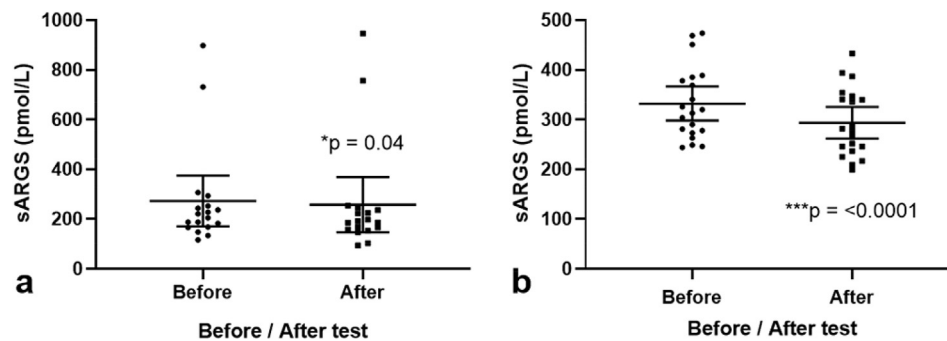


Fig. 3. Acute changes were seen in sARGs following short high intensity exercise. Absolute sARGs concentrations before and after HR_{max}-test in OA (a) and healthy subjects (b) are plotted. Paired *t*-test was applied on 20 pairs in both groups.

addition, based on the dynamics of sARGs observed in OA subjects, we suggest that sARGs is further evaluated as a biomarker in OA e.g. as a predictor of structural progression during a longer period of time (years). If found to be associated with risk of progression, sARGs used in a standardized exercise model could be valuable in early evaluation of DMOADs e.g. for evaluation of ADAMTS-5 monoclonal antibodies [34]. However, this needs further investigation.

4.2. Healthy subjects

sARGs decreased after the HR_{max}-test and after the running intervention in healthy subjects. This is in line with the weaker trends of initial decrease in the OA group and the significant decrease following the HR_{max}-test in the OA group. The reason for this transient drop is unknown, but it may be due to redistribution, metabolism, capillary leakage or a combination as a consequence of the cardiovascular stress. Another speculative explanation may be that acute intra-articular anti-inflammatory effects, which are known to be associated with exercise [17], temporarily reduce the activity of inflammation-sensitive proteases, such as MMPs and ADAMTS [35]. sARGs increased from baseline during rest and no considerable increase was observed following either exercise intervention. The findings were surprising in the context of a previous studies where dose-response relationship was observed between COMP and joint load [9], and proteoglycan fragment levels increase [36, 37].

The ICC of 0.77 in healthy subjects indicated a slight interday variability in sARGs in young healthy subjects. This interday variation, however, may have been negatively affected by only a few specific subjects, as most ARGs results were similar between the measured baseline visits. This could be a result of physical activity performed

between visits, but as no measure of background physical activity was collected, the exact reason for this phenomenon is unknown.

The negative correlation between age and baseline sARGs may reflect changes in synthesis rates of aggrecan, which have previously been found to decline with age [38]. A component here may likely be the closing of the chondral epiphyseal growth plates, which occurs in early adulthood [39]. This correlation is opposite of that seen in OA, which supports a fundamental difference in the significance of sARGs as a biomarker in subjects with and without OA, or a bi-phasic association as a function of age. However, it is important to highlight that the age-ranges in the two groups did not overlap, and thus direct comparison cannot be made.

4.3. The model

Prior to the current study no standardized model to investigate the acute impact of exercise on cartilage has been described. In a single study on OA patients, the chondroprotective effect of glucosamine was tested in an exercise model. Serum COMP decreased significantly compared to ibuprofen and placebo over 12-weeks of lower limb strength training [40]. The study indicate that a model combining cartilage load and biomarkers may be useful in assessing potential chondroprotective drugs. Our proposed model is intended to induce standardized loading to major joints at comparable cardiovascular stress, in order to measure the impact on cartilage using biochemical markers.

In studies investigating acute biomarker changes, it is standard procedure to have the subjects rest in the follow-up sample period, in order to detect the isolated effect of the exercise-intervention, but since complete rest itself is an intervention, which affects the biomarkers, most often in the opposite direction, complete rest may act as a partial counter-interaction dampening the exercise-induced response, potentially

Table 2

Group (n)	Mean absolute change (pmol/L (SD))					Mean proportional change (%) (SD)					Change from corresponding resting sample (%) (SD)			
	30 min	60 min.	120 min.	180 min.	24 h	30 min	60 min.	120 min.	180 min.	24 h	30 min	60 min	120 min	180 min.
Cycling (OA) (19)	-3.9 (23.3)	1.36 (21.0)	3.2 (25.0)	8.6 (27.9)	-5.9 (47.1)	-1.6 (5.7)	1.9 (6.5)	1.0 (7.4)	2.7 (8.5)	0.1 (10)	0.8 (15)	5.4 (15)	5.5 (20)	5.6 (18)
Running (OA) (14)	-7.1 (27.9)	2.9 (17.0)	2.9 (14.2)	8.5 (19.8)	2.5 (20.9)	-2.3 (7.0)	1.5 (5.5)	1.7 (5.4)	4.8 (9.1)	1.5 (7.8)	-0.3 (12)	5.2 (13)	5.2 (9.7)	6.7 (12)
Walking (OA) (5)	19.4 (42.1)	-21.2 (43.3)	-17.0 (55.9)	-23.0 (53.1)	19.4 (17.2)	1.7 (3.9)	-1.2 (5.3)	0.4 (6.6)	-2.0 (5.5)	-4.6 (4.5)	0.9 (9.4)	-1.6 (12)	1.4 (10)	-2.8 (12)
Resting (OA) (19)	-2.2 (28.1)	0.1 (29.4)	-1.6 (37.2)	6.7 (56.3)	N. a.	-0.6 (8.3)	-2.8 (8.0)	-2.1 (7.5)	-0.2 (10)	N. a.	N. a.	N. a.	N. a.	N. a.
Cycling (Healthy) (20)	-3.6 (31.1)	-1.8 (29.9)	7.7 (29.7)	7.0 (31.9)	8.5 (42.8)	-0.7 (7.2)	-0.6 (6.9)	1.9 (6.9)	1.5 (7.4)	2.9 (7.4)	-1.5 (15)	-2.9 (19)	-0.2 (15)	3.8 (17)
Running (Healthy) (20)	-16.8 (23.7)	-5.8 (20.3)	-0.8 (25.2)	4.5 (26.4)	-0.7 (34.8)	-3.6 (5.5)	-1.0 (4.2)	0.5 (6.0)	2.0 (7.0)	-0.1 (8.8)	-0.5 (14)	0.3 (15)	2.8 (15)	2.1 (12)
Resting (Healthy) (20)	5.0 (32.0)	10.4 (29.8)	7.1 (27.9)	15.0 (41.0)	N. a.	0.6 (7.8)	2.5 (7.2)	1.4 (7.2)	3.5 (10)	N. a.	N. a.	N. a.	N. a.	N. a.

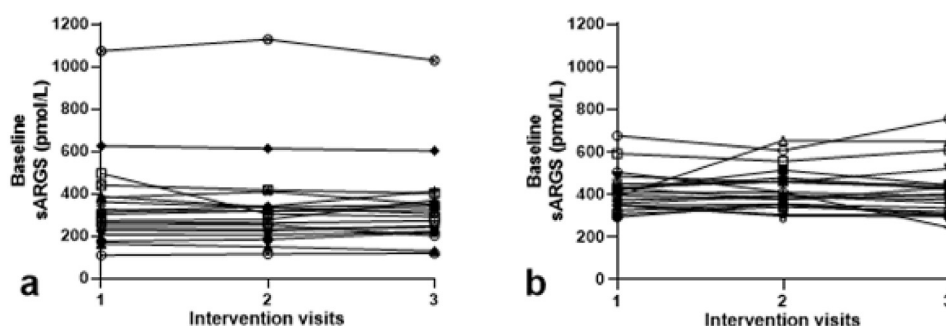


Fig. 4. a) sARGS interday variation in OA patients. b) sARGS interday variation in healthy subjects.

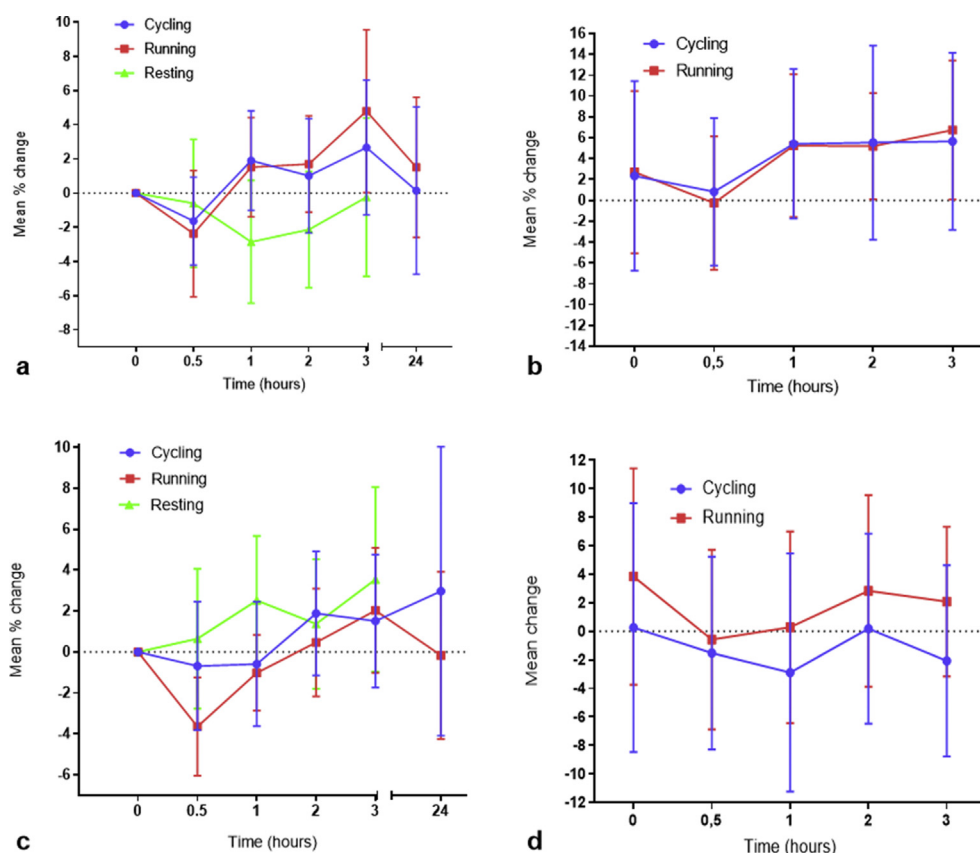


Fig. 5. Mean proportional change in sARGS from baseline, T_0 (immediately before exercise), in OA subjects ($n = 19$) (a–b) and in healthy subjects (c–d).

resulting in a reduction of the magnitude of the measured biomarker changes. A reason for this may be that the use of joints contributes to the movement of biomarker from its tissue of origin, e.g. the knee joint, to the bloodstream.

If refined, our method may facilitate identification of structural progressors [14] or ultimately assess response to structurally modifying treatment substantially quicker than in settings without evoked tissue turnover. However, the magnitude of sARGS response was relatively low, with an average change from baseline of approximately 5%. Development of the model might therefore include a combination of sARGS and other biomarkers of cartilage turnover to include not only ADAMTS-5 derived aggrecan turnover, but also other important joint tissue processes. As a minor finding, it was found that the HR_{max} -test may also be replaced with a simple HR_{max} -calculation, as we found very high concordance between the experimental and theoretical values in this study population.

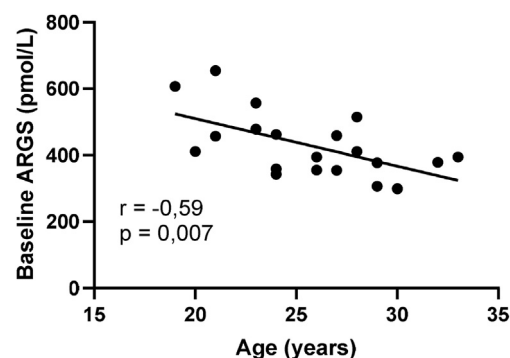


Fig. 6. a) Correlation between KL-grade and sARGs. Spearman's r reported. b) Correlation between age and baseline ARGs in OA subjects. Spearman's r reported.

4.4. Strengths and limitations

In the OA group age and male/female distribution was similar to that of typical clinical trial OA cohort, however BMI was lower, compared to the commonly described OA populations in clinical research. The characteristics of the healthy group are considered typical for the age group. The substantial differences in the age of the healthy and OA populations prohibits direct comparisons between the groups, but as the study was designed to compare within each of the two groups, and not across groups, it does not influence the main study results.

We only included subjects with primary OA. Thus, the results of this study does not necessarily reflect the pathological picture in secondary OA, such as post-traumatic OA.

As matched series of blood samples were taken during rest and exercise intervention was crossed over, subjects act as self-controls. The order of the exercise modalities was randomized. Prior to exercise interventions all subjects had completed HR_{max}-test in order to 1) enable standardization of the cardiovascular stress and 2) to neutralize the influence of potential plasma volume expansion after [41] the first exercise.

The feasibility of weight-bearing exercise in OA patients such as moderate intensity running, is not well described in the existing literature. Despite the presence of varying degree of joint damage and pain, the type, intensity, and duration of the proposed exercise modalities were considered feasible for the majority of the enrolled OA population, which, regardless of the results of the study, is interesting as it suggests good tolerability to moderately intense physical activity despite structural changes in the knee(s).

The most important limitation is the small sample size. The magnitude of the observed changes was relatively small, <10%, and it appears that a larger sample size could have contributed beneficially to the statistical rigor.

Aggrecan, and thus ARGS, is considered to be widely present in cartilage, but low amounts are present in veins. This may potentially contribute to sARGS levels, but as the current study design does not permit evaluation of tissue origin of aggrecan turnover, this is a limitation. Further studies may seek to establish the potential cardio-vascular contribution to sARGS levels.

The nature of this exercise-based model may have biased the study population towards subjects with generally better fitness. Thus the results may not apply alike to OA patients with different characteristics, but as all enrolled subjects met the radiographic criteria, the structural elements of the disease in the study are considered representative of other OA patients regardless of their physical fitness.

5. Conclusion

Minute acute increases in sARGS were observed in the hours following moderate exercise, possibly reflecting changes in cartilage turnover. sARGS sensitivity to physical activity is considered low and sARGS is considered a reproducible marker with stable individual levels from visit to visit.

The findings support the use of sARGS in clinical OA research, without a need to control for normal physical activity.

Author contributions

JJB, ARB, JRA and HBN designed the study. JJB and MB acquired the data. JJB, ARB, YH, ACBJ analyzed the data. JJB, ARB, MK and ALM interpreted the data. JJB, ABI and YH drafted the article. Critical revision and approval of the manuscript was performed by all authors.

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Declaration of Competing Interest

JJB received funding from Nordic Bioscience and the Danish Research Fund. HBN and YH are full-time employees of Nordic Bioscience. JRA, MK, ACBJ and ARB are full-time employees and shareholders of Nordic Bioscience. MB and ALM have no conflicts of interest.

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